The Concise Oxford Dictionary

TENTH EDITION

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Enclosure 1 - 2 pages

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– ORIGIN Fr., lit. 'gone by', past part. of *passer.*

passed pawn ● n. Chess a pawn that no enemy pawn can stop from queening.

passel /'pas(ə)l/ ● n. informal, chiefly US a large group: *a passel of journalists.*
– ORIGIN C19: representing a pronunc. of PARCEL.

passementerie /'pasm(ə)ntri/ ● n. decorative textile trimming consisting of gold or silver lace, gimp, or braid.
– ORIGIN C17: from Fr., from *passement* 'gold lace'.

passenger ● n. 1 a traveller on a public or private conveyance other than the driver, pilot, or crew. 2 a member of a team who does very little effective work.
– ORIGIN ME: from the OFr. adj. *passager* 'passing, transitory', from *passage* (see PASSAGE¹).

passenger mile ● n. one mile travelled by one passenger, as a unit of traffic.

passenger pigeon ● n. a migratory long-tailed North American pigeon, hunted to extinction by 1914. [*Ectopistes migratorius.*]

passepartout /,paspɑː'tuː, ,pɑːs-/ ● n. 1 a simple picture frame consisting of a piece of glass and a sheet of card (or two pieces of glass) taped together at the edges. 2 adhesive tape used in making such a frame. 3 archaic a master key.
– ORIGIN C17: from Fr., lit. 'passes everywhere'.

passer-by ● n. (pl. **passers-by**) a person who happens to be going past something, especially on foot.

passerine /'pasəraɪn, -riːn/ Ornithology ● adj. denoting birds of a large order (Passeriformes) distinguished by having feet adapted for perching and including all songbirds. ● n. a passerine bird.
– ORIGIN C18: from L. *passer* 'sparrow' + -INE¹.

pas seul /pɑː 'sɜːl/ ● n. a dance for one person.
– ORIGIN Fr., lit. 'single step'.

passible /'pasɪb(ə)l/ ● adj. Christian Theology capable of feeling or suffering.
– DERIVATIVES **passibility** n.
– ORIGIN ME: from OFr., from late L. *passibilis*, from L. *pass-, pati* 'suffer'.

passim /'pasɪm/ ● adv. (of references) at various places throughout the text.
– ORIGIN L., from *passus, pandere* 'scatter'.

passing ● adj. 1 going past. 2 (of a period of time) going by. 3 carried out quickly and lightly: *a passing glance.* ● n. 1 the passage of something, especially time. 2 the action of kicking, hitting, or throwing a ball to another team member during a sports match. 3 the end of something. ▸ euphemistic a person's death.
– PHRASES **in passing** briefly and casually.
– DERIVATIVES **passingly** adv.

passing bell ● n. a bell rung immediately after a death as a signal for prayers.

passing note ● n. Music a note not belonging to the harmony but interposed to secure a smooth transition.

passing shot ● n. Tennis a shot aiming the ball beyond and out of reach of one's opponent.

passion ● n. 1 strong and barely controllable emotion. ▸ an outburst of such emotion. 2 intense sexual love. 3 an intense enthusiasm for something. 4 **(the Passion)** the suffering and death of Jesus. ▸ a musical setting of this.
– DERIVATIVES **passional** adj. (rare). **passionless** adj.
– ORIGIN ME: from OFr., from late L. *passio(n-)*, from L. *pati* 'suffer'.

passionate ● adj. showing or caused by passion.
– DERIVATIVES **passionately** adv. **passionateness** n.

passion flower ● n. an evergreen climbing plant of warm regions, with a complex flower said to suggest things associated with Christ's Crucifixion. [Genus *Passiflora.*]

Passion Sunday ● n. the fifth Sunday in Lent.

Passiontide ● n. the last two weeks of Lent.

Passion Week ● n. 1 the week between Passion Sunday and Palm Sunday. 2 older name for HOLY WEEK.

passivate /'pasɪveɪt/ ● v. [usu. as adj. **passivated**] make (a metal or other substance) unreactive by coating or otherwise altering its surface.
– DERIVATIVES **passivation** n.

passive ● adj. 1 accepting or allowing what happens or what others do, without active response or resistance. 2 Grammar denoting a voice of verbs in which the subject undergoes the action of the verb (e.g. *they were killed* as opposed to *he killed them*). The opposite of ACTIVE. 3 denoting a circuit or device containing no source of energy or electromotive force. ▸ (of radar or a satellite) receiving or reflecting radiation rather than generating its own signal. 4 Chemistry unreactive because of a thin inert surface layer of oxide. ● n. Grammar a passive form of a verb.
– DERIVATIVES **passively** adv. **passiveness** n. **passivity** n.
– ORIGIN ME: from L. *passivus*, from *pass-, pati* 'suffer'.

passive immunity ● n. Physiology short-term immunity resulting from the introduction of antibodies from another person or animal.

passive resistance ● n. non-violent opposition to authority, especially a refusal to cooperate with legal requirements.

passive smoking ● n. the involuntary inhaling of smoke from other people's cigarettes, cigars, or pipes.

passivize (also **-ise**) ● v. Grammar convert into the passive.
– DERIVATIVES **passivizable** adj. **passivization** n.

pass key ● n. 1 a key given only to those who are officially allowed access. 2 a master key.

pass laws ● pl. n. laws formerly in operation in South Africa controlling the rights of black people to residence and travel.

Passover ● n. the major Jewish spring festival, commemorating the liberation of the Israelites from Egyptian bondage.
– ORIGIN from *pass over*, with ref. to the exemption of the Israelites from the death of their firstborn (Exod. 12).

passport ● n. an official document issued by a government, certifying the holder's identity and citizenship and entitling them to travel abroad under its protection.
– ORIGIN C15 (denoting authorization to depart from a port): from Fr. *passeport*, from *passer* 'to pass' + *port* 'seaport'.

password ● n. a secret word or phrase used to gain admission to something.

past ● adj. 1 gone by in time and no longer existing. ▸ recently elapsed: *the past twelve months.* 2 Grammar (of a tense) expressing a past action or state. ● n. 1 (usu. **the past**) a past period or the events in it. ▸ a person's or thing's history or earlier life: *the country's colourful past.* 2 Grammar a past tense or form of a verb. ● prep. 1 to or on the further side of. 2 in front of or from one side to the other of. 3 beyond in time; later than. 4 no longer capable of. 5 beyond the scope of. ● adv. 1 so as to pass from one side of something to the other. 2 used to indicate the passage of time: *a week went past.*
– PHRASES **not put it past someone** believe someone to be capable of doing something wrong or rash. **past it** informal too old to be any good at anything.
– DERIVATIVES **pastness** n.
– ORIGIN ME: var. of *passed*, past part. of PASS¹.

pasta ● n. dough extruded or stamped into various shapes (e.g. spaghetti, lasagne) for cooking in boiling water and eating, typically with a savoury sauce.
– ORIGIN C19: from Ital., lit. 'paste'.

paste ● n. 1 a thick, soft, moist substance, typically produced by mixing dry ingredients with a liquid. 2 an adhesive of this kind. 3 a mixture of kaolin and water, of low plasticity, used for making porcelain. 4 a hard vitreous composition used in making imitation gems. ... preparation in the shape of a small square', prob. from Gk *pastē*, (pl.) *pasta* 'barley porridge', from *pastos* 'sprinkled'.

pasteboard ● n. thin board made by pasting together sheets of paper.

pastel ● n. 1 a crayon made of powdered pigments bound with gum or resin. ▸ a work of art created using pastels. 2 a soft and delicate shade of a colour. ● adj. of or denoting such a shade: *pastel blue curtains.*
– DERIVATIVES **pastellist** (also **pastelist**) n.
– ORIGIN C17: via Fr. from Ital. *pastello*, dimin. of *pasta* 'paste'.

pastern /'past(ə)n/ ● n. the sloping part of a horse's or other animal's foot between the fetlock and the hoof.
– ORIGIN ME: from OFr. *pasturon*, from *pasture*, orig. 'strap for hobbling a horse'.

paste-up ● n. a document prepared by combining and pasting various sections on a backing.

pasteurellosis /,pɑːstərɛ'ləʊsɪs, ,pastərɛ'ləʊsɪs/ ● n. a bacterial infection commonly affecting animals and sometimes transferred to humans through bites and scratches.
– ORIGIN C20: from Fr. *pasteurellose*, from mod. L. *Pasteurella* genus name of the bacterium responsible (named after the C19 Fr. chemist and bacteriologist Louis *Pasteur*) + -OSIS.

pasteurize /'pɑːstʃəraɪz, -stjə-, 'pas-/ (also **-ise**) ● v. [often as adj. **pasteurized**] subject (milk, wine, etc.) to a process of partial sterilization, especially by heating.
– DERIVATIVES **pasteurization** n. **pasteurizer** n.

pasticcio /pa'stɪtʃəʊ/ ● n. (pl. **-os**) another term for PASTICHE.
– ORIGIN from Ital.

pastiche /pa'stiːʃ/ ● n. an artistic work in a style that imitates that of another work, artist, or period. ● v. create a pastiche of (an artist or work).
– DERIVATIVES **pasticheur** n.
– ORIGIN C19: from Fr., from Ital. *pasticcio*, based on late L. *pasta* 'paste'.

pastille /'past(ə)l, -tɪl/ ● n. 1 a small sweet or lozenge. 2 a small pellet of aromatic paste burnt as a perfume or deodorizer.
– ORIGIN C17: from Fr., from L. *pastillus* 'little loaf, lozenge', from *panis* 'loaf'.

pastime ● n. an activity that someone does regularly for enjoyment; a hobby.
– ORIGIN C15: from PASS¹ + TIME, translating Fr. *passe-temps.*

pastis /'pastɪs, pa'stiːs/ ● n. (pl. same) an aniseed-flavoured aperitif.
– ORIGIN from Fr.

past master ● n. an experienced person particularly skilled at a specified activity.

pastor /'pɑːstə/ ● n. a minister in charge of a Christian church or congregation, especially in some non-episcopal churches. ● v. be the pastor of.
– DERIVATIVES **pastorate** n. **pastorship** n.
– ORIGIN ME: from Anglo-Norman Fr. *pastour*, from L. *pastor* 'shepherd', from *past-, pascere* 'feed, graze'.

pastoral /'pɑːst(ə)r(ə)l/ ● adj. 1 used for or relating to the farming or grazing of sheep or cattle. 2 portraying country life, especially in an idealized or romanticized form. 3 of or relating to the giving of spiritual guidance. 4 of or denoting a teacher's responsibility for the general well-being of pupils or students. ● n. a pastoral poem, picture, etc.
– DERIVATIVES **pastoralism** n. **pastorally** adv.

pastorale /,pastə'rɑːl/ ● n. (pl. **pastorales** or **pastorali** /-liː/) 1 a slow instrumental composition in compound time, usually with drone notes in the bass. 2 a simple musical play with a rural subject.
– ORIGIN C18: from Ital., lit. 'pastoral'.

pastoralist ● n. 1 (especially in Australia) a sheep or ... typically served in thin slices.
– ORIGIN from Yiddish.

pastry ● n. (pl. **-ies**) 1 a dough of flour, fat, and water, used as a base and covering in baked dishes such as pies. 2 an item of food consisting of sweet pastry with a cream, jam, or fruit filling.
– ORIGIN ME: from PASTE, influenced by OFr. *pastaierie.*

pastry cream ● n. a thick, creamy custard used as a filling for cakes or flans.

pasturage ● n. 1 land used for pasture. 2 the occupation or process of pasturing animals.

pasture ● n. 1 land covered mainly with grass, suitable for grazing cattle or sheep. 2 grass and herbage growing on such land. ● v. put (animals) to graze in a pasture.
– PHRASES **pastures new** somewhere offering new opportunities. [suggested by 'Tomorrow to fresh woods and pastures new' (Milton's *Lycidas*).] **put out to pasture** force to retire.
– ORIGIN ME: from OFr., from late L. *pastura* 'grazing', from *past-, pascere* 'graze'.

pasty¹ /'pasti/ (also **pastie**) ● n. (pl. **-ies**) chiefly Brit. a folded pastry case filled with seasoned meat and vegetables.
– ORIGIN ME: from OFr. *paste(e)*, based on late L. *pasta* 'paste'.

pasty² /'peɪsti/ ● adj. (**-ier, -iest**) 1 of or like paste. 2 (of a person's face) unhealthily pale.
– DERIVATIVES **pastiness** n.

Pat ● n. Brit. informal, often offensive an Irishman.
– ORIGIN C19: abbrev. of the given name *Patrick.*

Pat. ● abbrev. Patent.

pat¹ ● v. (**patted, patting**) 1 touch quickly and gently with the flat of the hand. 2 mould or position with gentle taps. ● n. 1 a light stroke with the hand. 2 a compact mass of soft material.
– PHRASES **a pat on the back** an expression of congratulation or encouragement.
– ORIGIN ME: prob. imitative.

pat² ● adj. simple and somewhat glib or unconvincing: *a pat answer.* ● adv. conveniently or opportunely.
– PHRASES **have something off** (or **down**) **pat** have something memorized perfectly. **stand pat** chiefly N. Amer. 1 stick stubbornly to one's opinion or decision. 2 (in poker and blackjack) retain one's hand as dealt.
– DERIVATIVES **patly** adv. **patness** n.
– ORIGIN C16: rel. to PAT¹; appar. orig. 'as if with a pat'.

pataca /pə'tɑːkə/ ● n. the basic monetary unit of Macao, equivalent to 100 avos.
– ORIGIN from Sp. and Port.

pat-a-cake ● n. a children's game with gentle patting in time to the words of a rhyme.

patagium /,patə'dʒaɪəm/ ● n. (pl. **patagia** /-'dʒaɪə/) 1 Zoology a membrane between the forelimbs and hindlimbs on each side of a bat or gliding mammal. 2 Entomology a lobe that covers the wing joint in many moths.
– ORIGIN C19: from L., denoting gold edging on a Roman lady's tunic, from Gk *patageion.*

Patagonian /,patə'gəʊnɪən/ ● n. a native or inhabitant of the South American region of Patagonia. ● adj. of or relating to Patagonia.

pataphysics /,patə'fɪzɪks/ ● pl. n. [usu. treated as sing.] a supposed branch of philosophy concerned with an imaginary realm additional to metaphysics.
– ORIGIN 1940s: from Gk *ta epi ta metaphusika*, lit. 'the (works) imposed on the metaphysics', introduced by the Fr. absurdist writer Alfred Jarry.

patas monkey /pə'tɑː/ ● n. a central African guenon with reddish-brown fur, a black face, and a white moustache. [*Erythrocebus patas.*]
– ORIGIN C18: *patas* from Senegalese Fr., from Wolof *pata.*

Patau's syndrome /'pataʊz/ ● n. Medicine a congenital chromosomal disorder resulting in brain, heart, and kidney defects, usually fatal soon after birth.
– ORIGIN 1960s: named after the Ger. physician Klaus

3rd Edition

Basic Histology

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Enclosure 2 - 2 pages

morphologic characteristics of collagen fibers are better studied in spread preparations than in histologic sections. Mesentery is frequently used for this purpose, for when spread on a slide, it is sufficiently thin to be stained and examined under the microscope. Mesentery is composed of a central portion of connective tissue lined on both surfaces by a simple squamous epithelium, the mesothelium. The collagen fibers in a spread preparation appear as elongated and tortuous cylindric structures. Their endings merge with other components of the tissue and cannot be seen. The diameter of collagen fibers varies from 1–20 μm (Fig 5–1). These fibers are longitudinally striated and are composed of **fibrils** with a diameter of 0.2–0.5 μm. The diameter of the fibers depends on the number of fibrils they contain.

The electron microscope also shows that each fibril is made up of finer filaments whose dimensions cannot be resolved by the light microscope. Collagen fibrils present a characteristic cross-banding with a periodicity of 64 nm (Fig 5–2). Each fibril presents a sequence of dark and light bands. The dark bands retain more of the stain used in electron microscopic studies because they have more free chemical radicals than the light bands (Fig 5–3). In addition to the typical 64-nm banding fibrils, collagen fibrils with a periodicity of approximately 250 nm exist in the connective tissue of the eye and in the cartilage of elderly people. These fibrils are called **fibrous long space** collagen.

Seen in the light microscope, collagen fibers are acidophilic; they stain pink with eosin, blue with Mallory's trichrome stain, and green with Masson's trichrome stain.

The principal amino acids composing collagen are glycine (33.5%), proline (12%), and hydroxyproline (10%). The remainder is made up of other amino acids, although it is interesting to note that collagen is very low in sulfated amino acids and in tyrosine. It is the only protein containing an appreciable amount of hydroxyproline. (Elastin is the only other substance that contains hydroxyproline, although in very small quantities.) The amount of collagen in a tissue can therefore be determined by measurement of the hydroxyproline content. Another amino acid unique to collagen is hydroxylysine. Collagen is the most abundant protein of the human body, representing 30% of total body proteins.

The protein subunit that polymerizes to form collagen fibrils is an elongated molecule called **tropocollagen**, which measures 280 nm in length and 1.5 nm in width. Tropocollagen consists of 3 polypeptide chains (Fig 5–4). In type I collagen, 2 of these peptide chains are alike (alpha-1) and differ from the third (alpha-2) in their amino acid sequence. The tropocollagen molecule is asymmetric, ie, each end has a different chemical composition. These molecules are the building blocks from which fibrils are formed. The transverse striation of the collagen fibrils is determined by the overlapping arrangement of the subunit tropocollagen molecules (Fig 5–3).

More detailed studies on the chemical structure of collagen have revealed that the amino acid composition of the alpha-1 chain varies according to its location in the body. The most widespread collagen, known as type I, consists of 2 alpha-1 (type I) chains and one alpha-2 chain. This collagen appears in the dermis of the skin, tendons, bone, teeth, and virtually all other connective tissues. Type II colla-

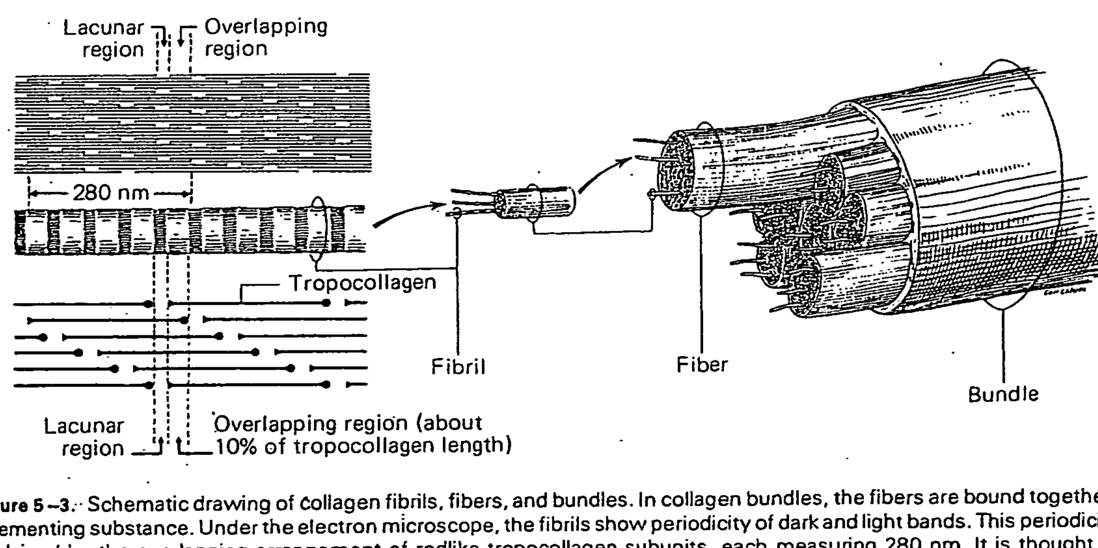

Figure 5–3. Schematic drawing of collagen fibrils, fibers, and bundles. In collagen bundles, the fibers are bound together by a cementing substance. Under the electron microscope, the fibrils show periodicity of dark and light bands. This periodicity is explained by the overlapping arrangement of rodlike tropocollagen subunits, each measuring 280 nm. It is thought that tropocollagen molecules are organized in a step-wise arrangement that produces lacunar and overlapping regions. Lacunar regions contain more stain (uranyl acetate, phosphotungstic acid) and appear dark.

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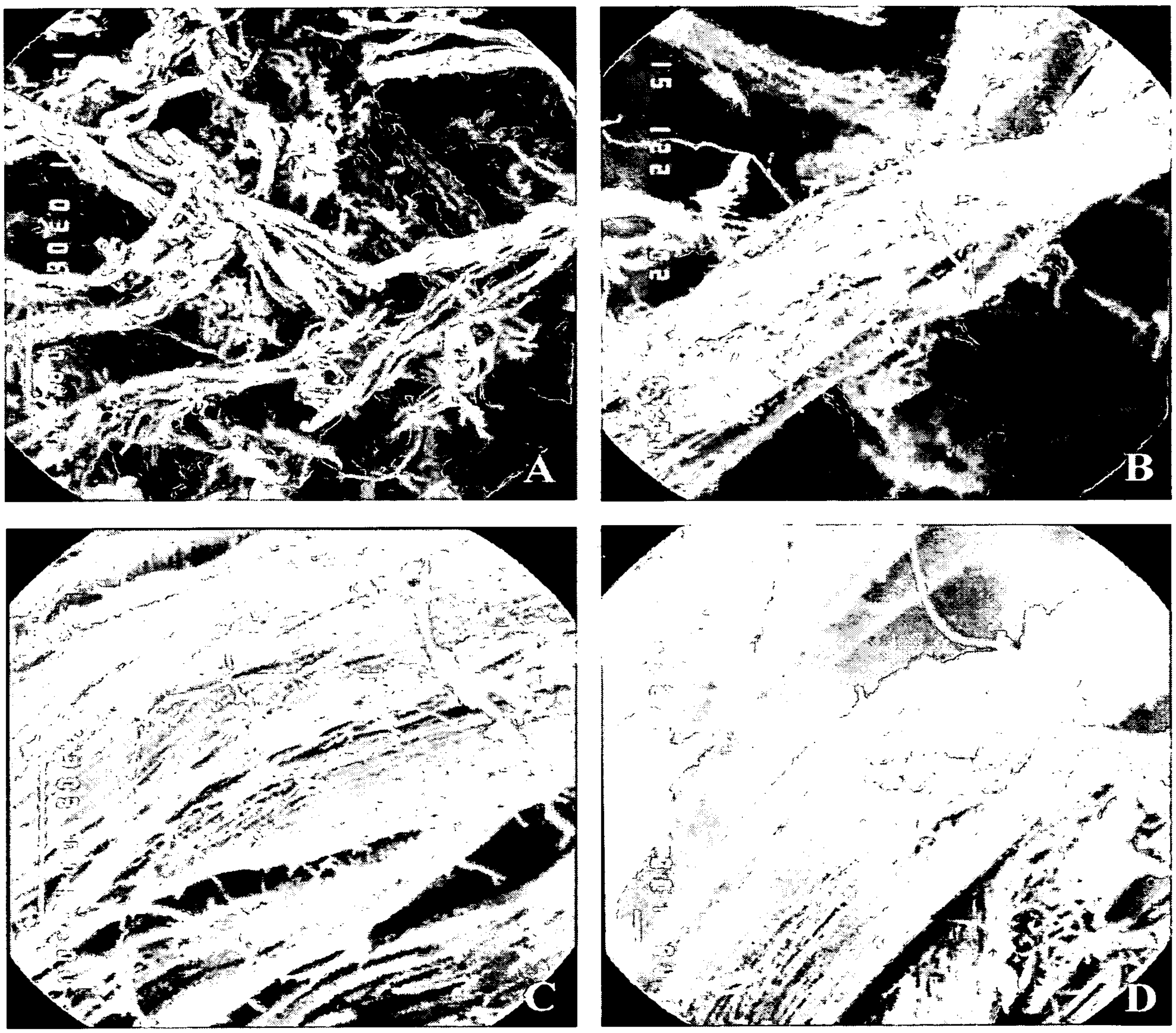
ELECTRON MICROGRAPH OF COMMINUTED COLLAGEN



Magnification

A = 200x C = 4400x

B = 1200x D = 4400x

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Enclosure 3 - 1 page

COLLAGEN

Volume III
Biotechnology

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Enclosure 4 - 5 pages

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I. INTRODUCTION

Collagen derived from bovine hide has been used as a biomaterial for nearly two decades.[1-4] In most applications, collagen is formed into solid articles, such as tubes, sheets, and threads. These are designed for use as blood vessels, burn dressings, and sutures.[1-4] Recently, however, collagen has also been prepared in a fluid form[5-9] and used to correct contour deficiencies of the dermis, soften dermal scars,[10-11] and augment lesions of the esophageal sphincter[12] and vocal cord.[13] Because it is a fluid, the collagen can be delivered to the site by injection. This mode of delivery is preferable to invasive surgical techniques, and the treatment can be done on an out-patient basis. Once *in situ*, the physical requirements for such implant materials change. It is no longer desirable that they be fluid. Instead, they should be elastic solids that remain at the site of placement and yield to applied stresses in a manner similar to surrounding tissue. Fortunately, the viscoelastic behavior of aqueous suspensions of fibrillar collagen spans both fluid and solid domains, and the transition from one domain to the other can be controlled in part by the level of imposed stress. Another important control parameter is temperature; when prepared in the appropriate form, the fluidity of fibrillar collagen suspensions can be decreased significantly by elevating the temperature. It is one of the goals of this chapter to describe these properties in detail and relate them to the underlying molecular structure.

In addition to fulfilling the physical requirements mentioned above, a successful implant must also be biocompatible. It should not induce a foreign body response, nor should it be highly immunogenic. An ideal implant may also permit colonization by normal connective tissue cells and even promote deposition of new host connective tissue. In terms of duration, a permanent implant may be desired for some indications. However, if the implant does not age in the same manner as the surrounding tissue, or if other long-term changes occur, a gradual decline in implant volume may be preferred with periodic reimplantation, if appropriate. The relationship of collagen implant chemistry and fiber morphology to these aspects of biocompatibility will be considered in this chapter.

II. CHARACTERIZATION OF INJECTABLE COLLAGEN

A. Preparation of ZYDERM® Collagen Implant (ZCI)

ZCI is a sterile suspension of bovine fibrillar collagen in 0.02 *M* sodium phosphate, 0.13 *M* sodium chloride, and 0.3% lidocaine, pH 7.2. The fibrils are precipitated from a soluble collagen intermediate (SCI), harvested, and resuspended in the final buffer at two protein concentrations: 35 ± 5 and 65 ± 5 mg/mℓ.[6] SCI is isolated from bovine corium: the hide is softened, depilated, and then comminuted and pepsin digested. The solution is then purified and concentrated to form a 3 mg/mℓ solution of telopeptide-poor bovine collagen in dilute aqueous HCl, pH 2.[6]

B. Other Injectable Forms of Collagen

There are references in the literature[14,15] to potentially injectable forms of collagen, which are not fibrillar. Miyata et al.[14] have prepared methylated and succinylated collagen, which can be precipitated at pH 9 and 4, respectively. When such precipitates are taken to pH 7 to 8, they "redissolve" to form transparent, viscous, gel-like materials. Bruns and Gross[15] subjected dilute acidic collagen solutions to high-speed centrifugation, again yielding a concentrated, viscous gel or fluid. This latter material is not at physiological pH, and would require addition of inhibitors of fibrillogenesis, such as arginine, to maintain the nonfibrillar state when adjusted to pH 6 to 8. Neither of these materials has been specifically recommended as a tissue implant, but both appear to possess the requisite physical characteristics for such an application. There is clear collagen solution at 20 mg/mℓ of undisclosed com-

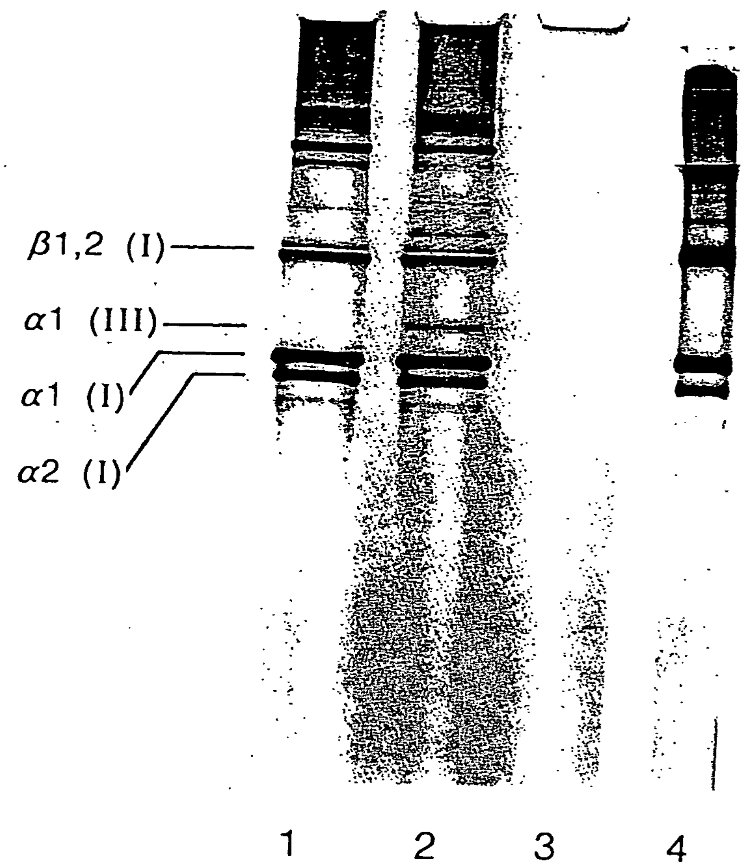

FIGURE 1. SDS polyacrylamide gel electrophoresis of the pepsin-solubilized bovine corium collagen soluble collagen intermediate, (SCI). Electrophoresis was performed according to the method of Laemmli[17] as modified by Studier[18] employing a 4% stacking gel and a 4 to 15% gradient separation gel. Lane 1, 4 μg of SCI subjected to electrophoresis under nonreducing conditions; lane 2, 4 μg of SCI subjected to electrophoresis under interrupted reducing conditions (see α1 [III]); lane 3, 4, μg of SCI which had been preincubated with bacterial collagenase (Type III, Advance Biofactures Corp., Lynbrook, N.Y.) at concentrations of 0.5 mg/mℓ and 82.5 units/mℓ, respectively, for 37°C for 4 hr and then subjected to electrophoresis under nonreducing conditions; lane 4, ZCI resolubilized in acid and tested as in lane 1 (electrophoresis of this sample was carried out in a separate experiment). (From McPherson, J. M. et al., *Collagen Rel. Res.*, 5, 119, 1985. With permission.)

position sold for human use in some countries under the name Koken® Atelocollagen (Koken Ltd., Tokyo, Japan). The performance of these materials relative to fibrillar implants is unknown.

C. Characterization of ZCI: The Solubilized Form, SCI

The purity of the highly purified, pepsin-solubilized, SCI has been demonstrated by collagenase digestion (Advance Biofactures Type III, Lynbrook, N.Y.) and examination by SDS polyacrylamide gel electrophoresis in conjunction with a highly sensitive silver staining technique[16] (Figure 1). Electrophoretic analysis[17,18] of this material after denaturation indicates that it is largely Type I collagen and contains less than 5% Type III collagen (Figure

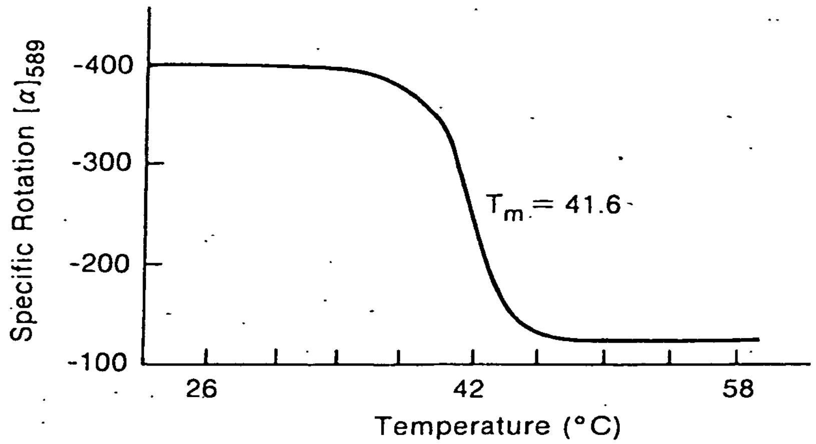

FIGURE 2. Melting curve of resolubilized ZCI. ZCI was dialyzed overnight at 4°C against 5 m*M* acetic acid to give a clear solution and diluted to 1 to 2 mg of protein per milliliter with 5 m*M* acetic acid. The thermal denaturation curve was recorded at 589 nm in a polarimeter (JASCO model DIP-140) equipped with a water-jacketed cell. The cell was connected to a water bath which was programmed to scan from 22 to 52°C at 0.4°C/min. The actual sample temperature was corrected, if necessary, by measurements made by a thermocouple mounted in the cell. At the beginning of each experiment, the specific rotation, $[\alpha]_{589}$, was set to −400°C. This value for $[\alpha]_{589}$ of native collagen was the average observed for pepsinized bovine corium and rat tail tendon collagens whose concentrations had been determined by amino acid analysis. (From McPherson, J. M. et al., *Collagen Rel. Res.*, 5, 119, 1985 With permission.)

1). This analysis also shows that the starting material contains α, β, and γ components as well as higher aggregates. Fragments smaller than α chains represent approximately 5% of the total collagen as judged by scans of the SDS gels. Amino acid analysis of the collagen shows two residues of tyrosine per thousand residues, indicating 50 to 70% removal of telopeptides.[19] Most of the remaining tyrosine is presumably present in peptide fragments, derived from the nonhelical, telopeptide ends of the α chains that are still attached to the helical body of another molecule through lysine-derived covalent cross-links. Transmission electron microscopy, utilizing the rotary shadowing technique,[20] reveals that the polydispersity of bovine pepsin-treated collagen in acidic solution is approximately 80% monomeric and 13% dimeric, with some higher aggregates.[21] The remaining 7% consists of shortened fragments, which appear to be bimodal in size distribution, half being about one fourth and half being about three fourths the length of the native rod.[22] After precipitation in 0.02 *M* sodium phosphate at 17°C, and redissolution in acid, the content of fragments can be reduced to less than 0.5%[21,22] Heat denaturation experiments, using polarimetry[23] (see Figure 2), also show evidence for small amounts of nicked or fragmented helices.

D. Fibrillar Structure

Transmission electron microscopy reveals that ZCI is a highly polydisperse mixture of fibrils. The smallest fibrils are 5 to 10 nm in diameter, at least several microns in length, and make up approximately 90% (number frequency) of all fibrils. Intermediate diameter fibrils (about 50 nm in diameter) and large, banded fibrils (about 100 nm in diameter) make up the remainder and are more or less scattered throughout the mixture (Figure 3).[23] Fibril size distributions estimated from electron micrographs must be considered approximate, since fixation techniques may encourage fibril disassembly or obscure the smallest fibril classes. The mean fibril size in these preparations is much smaller than that usually reported

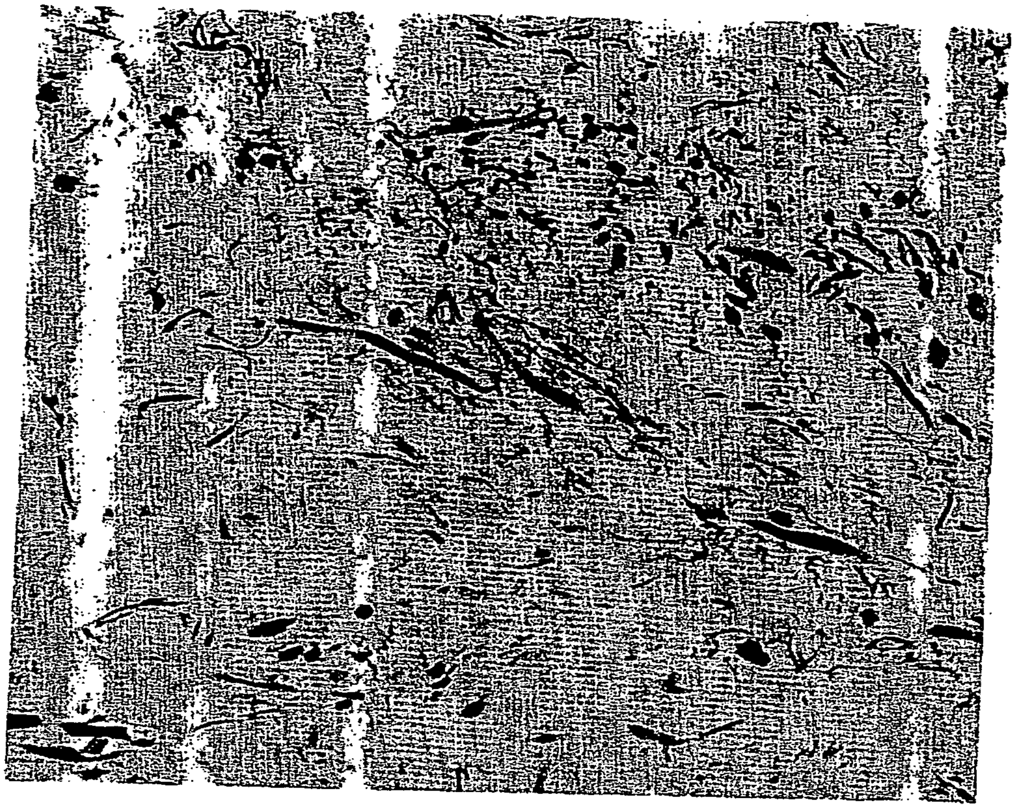

FIGURE 3. Electron microscopy of ZCI. ZCI samples (35 mg/mℓ) were fixed in 2% glutaraldehyde in 0.2 *M* cacodylate buffer, pH 7.3 at 4°C. Samples were washed and postfixed in 1% OsO_4 for 1½ hr at room temperature. Samples were then stained in 1.7% uranyl acetate, washed in veronal acetate buffer, dehydrated with ethanol, cleared with propylene oxide and embedded in maraglas. Thin sections, 50 to 70 nm, were prepared. Bar = 400 nm. (From McPherson, J. M. et al., *Collagen Rel. Res.*, 5, 119, 1985. With permission.)

in the literature, even for pepsin solubilized collagen.[24,25] This is presumably due to the fact that addition of salt and storage at 5°C causes partial disassembly of the initially precipitated fibrils.[26,27]

The polydisperse nature of fibril classes in ZCI is further revealed by differential scanning calorimetry (DSC).[28] Figure 4 presents the denaturation pattern of ZCI when heated at 10°C/min. Multiple denaturational transitions are observed at about 39, 44, 50, 53, and 57°C. Deconvolution of traces is required to reveal the weaker endotherms at 39, 50, and 57°C. Control tests at different heating rates and protein concentrations show that the multiple endotherms are characteristic of the material itself and not due to experimental artifacts. It is noteworthy that immediately upon precipitation at 17°C in 0.02 *M* sodium phosphate, the fibrillar samples exhibit a monodisperse pattern by DSC (T_m near 53°C),[28] but when salt is added and the sample is stored at 5°C, then the polydisperse pattern in DSC develops.

It has been proposed[28] that the heterogeneity seen by DSC of ZCI is due to sequential melting of distinct classes of fibrils, each with its own characteristic melting temperature (T_m). Three mechanisms that may play a role in stabilizing such distinct fibril classes are (1) variations in the level of intrafibril cross-linking (more cross-linked fibrils have higher T_m values), (2) fibrillar packing order, and (3) surface energy effects as a function of fibril diameter (larger diameter fibrils have higher T_m values). The latter mechanism has been